

[0125] Gates for use with the present invention may be simple gates, or mixing gates. Mixing gates are components that allow two volumes of liquid to be combined (e.g., mixed).

Vents

[0126] A vent is a structure that permits gas (e.g., air), such as gas displaced by the movement of liquids within component 201, to exit a channel while simultaneously limiting (e.g., preventing) liquid from exiting the channel. Vents thus allow component 201 to be vented so that pressure buildup does not inhibit desired movement of the liquids.

[0127] Typically, a vent is a hydrophobic vent and includes a layer of porous hydrophobic material (e.g., a porous filter such as a porous hydrophobic membrane, available from Osmonics) that defines a wall of the channel. As discussed hereinbelow, hydrophobic vents can be used to position a microdroplet of sample at a desired location within component 201.

[0128] Hydrophobic vents typically have a length of at least about 2.5 mm (e.g., at least about 5 mm, at least about 7.5 mm) along a channel. The length of a hydrophobic vent is typically at least about 5 times (e.g., at least about 10 times, at least about 20 times) larger than a depth of the channel within the hydrophobic vent. For example, in some embodiments, the channel depth within the hydrophobic vent is about 300 microns or less (e.g., about 250 microns or less, about 200 microns or less, about 150 microns or less).

[0129] The depth of the channel within the hydrophobic vent is typically about 75% or less (e.g., about 65% or less, about 60% or less) of the depth of the channel upstream and downstream of the hydrophobic vent. For example, in some embodiments the channel depth within the hydrophobic vent is about 150 microns and the channel depth upstream and downstream of the hydrophobic vent is about 250 microns.

[0130] A width of the channel within the hydrophobic vent is typically at least about 25% wider (e.g., at least about 50% wider) than a width of the channel upstream from the vent and downstream from the vent. For example, in an exemplary embodiment, the width of the channel within the hydrophobic vent is about 400 microns and the width of the channel upstream and downstream from the vent is about 250 microns.

Waste Chambers

[0131] Waste chambers are elements that can receive waste (e.g., overflow) liquid resulting from the manipulation (e.g., movement and/or mixing) of liquids within microfluidic component. Typically, each waste chamber has an associated air vent that allows gas displaced by liquid entering the chamber to be vented. An exemplary waste chamber is shown at 269 in FIG. 4.

System

[0132] Elements of component 201 are typically thermally actuated. Accordingly, in use, cartridge 200 is typically in communication with a heating element, such as an array of heat sources (e.g., resistive heat sources as exemplified in FIGS. 7-9), configured to operate the elements (e.g., valves, gates, actuators, and processing region) of microfluidic component 201. By 'in communication', is included to mean thermally associated, for example in thermal contact with a

heat source. In preferred embodiments, cartridge 200 is insertable into, and removable from, a cartridge receiving element in a system such as shown in FIG. 1. The heating element is in communication with the cartridge receiving element and is configured to heat one or more regions of the cartridge.

[0133] In some embodiments, the heat sources are operated by a computer operating system, which operates the device during use by communicating instructions to various control circuitry that is in communication with the heating element. The operating system includes a processor (e.g., a computer) configured to actuate the heat sources at specific times, according to a desired protocol. Processors configured to operate microfluidic devices are described in U.S. application Ser. No. 09/819,105, filed Mar. 28, 2001, which is incorporated herein by reference. In other embodiments, the heat sources are integral with the system itself.

[0134] Preferably, heat sources in the array of heat sources have locations that correspond to elements, such as actuators, gates, and valves, of microfluidic component 201.

Lyophilized Particles

[0135] Lyophilized reagent pellets 260 of bulk lysis chamber 264 include one or more compounds (e.g., reagents) configured to release polynucleotides from cells (e.g., by lysing the cells). For example, pellets can include one or more enzymes configured to reduce (e.g., denature) proteins (e.g., proteinases, proteases (e.g., pronase), trypsin, proteinase K, phage lytic enzymes (e.g., PlyGBS)), lysozymes (e.g., a modified lysozyme such as ReadyLyse), cell specific enzymes (e.g., mutanolysin for lysing group B streptococci)).

[0136] The pellets generally have a room temperature (e.g., about 20° C.) shelf-life of at least about 6 months (e.g., at least about 12 months). Liquid sample entering the bulk lysis chamber dissolves (e.g., reconstitutes) the lyophilized compounds.

[0137] Typically, pellets 264 have an average volume of about 35 microliters or less (e.g., about 27.5 microliters or less, about 25 microliters or less, about 20 microliters or less). In some embodiments, the particles have an average diameter of about 8 mm or less (e.g., about 5 mm or less, about 4 mm or less). In an exemplary embodiment the lyophilized pellets have an average volume of about 20 microliters and an average diameter of about 3.5 mm.

[0138] In some embodiments, pellets alternatively or additionally include components for retaining polynucleotides as compared to inhibitors. For example, pellets 260 can include multiple pellets surface modified with ligands, as discussed hereinabove. Pellets 260 can include enzymes that reduce polynucleotides that might compete with a polynucleotide to be determined for binding sites on the surface modified particles. For example, to reduce RNA that might compete with DNA to be determined, pellets 260 may include an enzyme such as an RNAase (e.g., RNAaseA ISC BioExpress (Amresco)).

[0139] In an exemplary embodiment, pellets 260 cells include a cryoprotectant. Cryoprotectants generally help increase the stability of the lyophilized particles and help prevent damage to other compounds of the particles (e.g., by preventing denaturation of enzymes during preparation and/